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CITATION:

Hama, Masaharu. The Use of Preserved Hematopoietic Tissues for Treatment of Mice Lethally Irradiated with Gamma Rays under High Dose Rate. (II) : Effect of Preserved Homologous Fetal Liver (Special Issue on Physical, Chemical and Biological Effects of Gamma Radiation, VII). Bulletin of the Institute for Chemical Research, Kyoto University 1966 ...

ISSUE DATE:

1966-03-31

URL:

<http://hdl.handle.net/2433/76109>

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# The Use of Preserved Hematopoietic Tissues for Treatment of Mice Lethally Irradiated with Gamma Rays under High Dose Rate. (III)

## Effect of Preserved Homologous Fetal Liver

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*Received January 10, 1966*

Mice were irradiated lethally with gamma rays under high dose rate and were treated with preserved homologous fetal liver. The homologous fetal liver was suspended in 15% glycerol-Tyrodé's solution or 10% dimethyl-sulphoxide TC199 solution and preserved at  $-80^{\circ}\text{C}$  as long as 360 days. The homologous fetal liver cells which were preserved as long as 360 days could protect the lethally irradiated mice.

### INTRODUCTION

Mice exposed to a lethal dose of X radiation survive acute irradiation damage when they are given a postirradiation inoculation of bone marrow or spleen cells<sup>1)</sup>. Inoculation of isologous bone marrow produces excellent recovery, while homologous bone marrow affords only a temporary recovery<sup>2)</sup>; the mice succumb after 21 or 30 days. The transient nature of this recovery following inoculation of homologous marrow has been a subject of extensive investigations from which two main theories have emerged. Makinodan and his coworkers<sup>3,4,5)</sup> have evidences which they feel support the host versus graft theory. However, Barnes and Loutit<sup>2)</sup> postulated an immune response of the graft versus host. There is an increasing body of evidences in support of this latter theory as an explanation of the cause of secondary disease and delayed death<sup>6-10)</sup>. If there exists a graft versus host reaction when adult bone marrow is used, fetal liver or spleen might not initiate such a reaction owing to its immunological nonreactivity or immaturity<sup>11-14)</sup>.

### MATERIALS AND METHODS

Mice. Dd/s and Na<sub>2</sub> strain mice supplied exclusively from the Kyoto University Animals Center were used as recipients and donors of the homologous fetal liver, respectively. The irradiated mice, after fetal liver transplantation or injection of only Tyrodé's solution, were kept in wooden boxes measuring 15×21×30cm. Eight to ten mice in a box were fed wheat supplemented with dried fish every other day and were given tap water *ad libitum*.

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Fetal liver cells suspensions. Isologous fetal livers (IFL) were obtained from the fetuses after the mating of Dd/s strain. The F<sub>1</sub> hybrids fetal livers (SFL) were obtained from the fetuses after the matings of female Dd/s strain and male Na<sub>2</sub> strain mice. The homologous fetal livers (HFL) were obtained from the matings of Na<sub>2</sub> strain mice. The donor tissue was obtained from fetuses of 15 to 19 days gestation. The gestational age of the fetus was carefully determined from the external features of the mouse embryo by the method of Gruneberg<sup>15)</sup>. The livers of male and female donors of the same age were pooled. Suspensions of pooled fetal livers in Tyrode's solution or TC 199 solution were prepared by gentle aspiration through a syringe. The suspensions were filtered through the surgical gauzes and the suspensions were passed through a small needle (1/5 : usually used for the tuberculin test<sup>14)</sup>). One fetal liver was suspended in 0.5cc of Tyrode's solution or TC 199 solution. Glycerol was added so as to make the final concentration of 15% V/V<sup>16~20)</sup> and dimethylsulphoxide (DMSO) was added to make the final concentration of 10% V/V<sup>21~25)</sup>. There were about forty million nucleated cells in one fetal liver. An ampoule containing 3.6cc of the fetal liver suspended in 15% glycerol-Tyrode's solution or 3.3cc of the fetal liver suspended in 10% DMSO-TC 199 solution was sealed with flame. The procedures were carried out under aseptic conditions. Each recipients, except the mice in the control group, was given nucleated fetal liver cells via the tail vein within 4 to 6 hours after irradiation. The mice in the control group were lethally irradiated without any further treatment or were injected intravenously 0.5ml of Tyrode's solution.

Freezing and thawing. Slow freezing<sup>18,20,27,28)</sup> means that the temperature was cooled at a rate of 1-2°C/min. from 0°C until -25°C and cooled to -80°C at a rate not exceeding 10°C/min. The method of slow freezing and fast thawing was described previously.

Irradiation. A Co<sup>60</sup> gamma-irradiation facility which belongs to the Institute for Chemical Research of Kyoto University was used in the experiments. This facility was described in detail<sup>29,30)</sup>. All recipients were exposed to a single LD<sub>90</sub>/14 days, LD<sub>100</sub>/21 days dose of total body irradiation of 900r which was obtained by the irradiation time of 27 sec. in October, 1962, and of 32 sec. in October, 1964.

Cell viability. As a screening test, the eosin staining method<sup>31)</sup> was used as described previously.

## RESULTS

### 1. Some Observations on the Basic Method of Fetal Liver Transplantation

Exp. 1. The experiment was carried out to protect the lethally irradiated mice with the fresh fetal liver which was suspended in Tyrode's solution. Table 1 shows the survival rates of irradiated mice treated with the isologous fetal liver (IFL), the F<sub>1</sub> hybrids fetal liver (SFL) and the homologous fetal liver (HFL). The survival rates in IFL treatment was as follows : 100% at 14 days, 100% at 30 days, 75% at 60 days and 75% at 120 days after irradiation. The survival rates in SFL treatment were as follows : 91% at 14 days, 78% at 21 days, 47% at 30

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Table 1. Survival rate of gamma-irradiated mice treated with HFL.

Cell number	No. of experiment	Number of survival Number of irradiated (at days)						% Survival (at days)				
		14	21	30	60	90	120	21	30	60	90	120
Control	44	2/162	0/162	—	—	—	—	0	—	—	—	—
IFL	2	19/19	18/18	16/16	12/16	12/16	12/16	100	100	75	75	75
SFL	4	22/24	18/23	11/23	7/23	4/23	3/23	78	47	30	17	13
5×10 <sup>6</sup> HFL	2	17/19	16/18	14/16	10/16	9/16	8/16	88	87	62	52	50
10×10 <sup>6</sup> HFL	2	18/22	13/22	9/21	4/20	3/20	2/20	59	42	20	15	10
20×10 <sup>6</sup> HFL	5	27/33	18/33	11/33	5/32	4/32	4/32	54	34	15	12	12

Control means the group of gamma-irradiated mice treated with only Tyrode's solution or nothing.

IFL means the group of gamma-irradiated mice treated with IFL when the number of nucleated cells injected was 20 million.

SFL means the F<sub>1</sub>-hybrids fetal liver transplantation when the number of nucleated cells injected was 20 million.

5×10<sup>6</sup> HFL means the HFL transplantation when the number of nucleated cells injected was 5 million.

10×10<sup>6</sup> HFL means the HFL transplantation when the number of nucleated cells injected was 10 million.

20×10<sup>6</sup> HFL means the HFL transplantation when the number of nucleated cells injected was 20 million.

days, 30% at 60 days, 17% at 90 days and 13% at 120 days. The survival rates in HFL treatment in which the number of nucleated cells injected was 5 million were as follows : 89% at 14 days, 88% at 21 days, 87% at 30 days, 62% at 60 days and 50% at 120 days. The survival rates in HFL treatment in which the number of nucleated cells injected was 10 million were as follows : 89% at 14 days, 59% at 21 days, 20% at 60 days and 10% at 120 days. The survival rates in HFL treatment in which the number of nucleated cells injected was 20 million were as follows : 81% at 14 days, 54% at 21 days, 34% at 30 days, 15% at 60 days and 12% at 120 days. The survival rates in IFL transplantation are excellent as in isologous bone marrow transplantation. Generally speaking, the HFL cells or

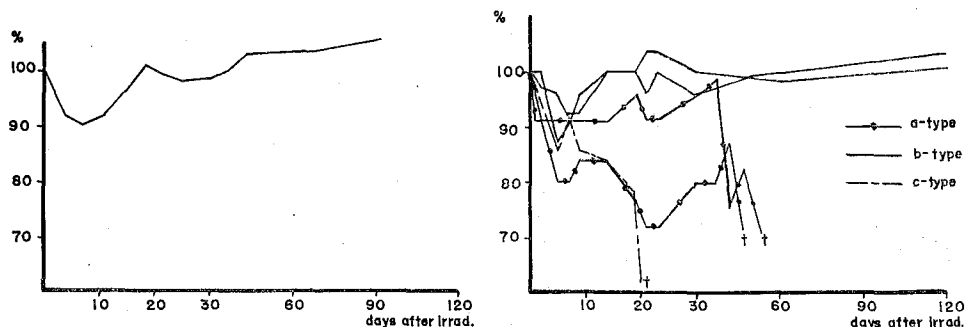


Fig. 1. Body weight changes in the mice irradiated lethally and treated with fetal liver. The number of nucleated cells injected was 20 million. They were suspended in Tyrode's solution. The left figure shows the changes in the mice treated with IFL. The right figure shows the changes in the mice treated with SFL.

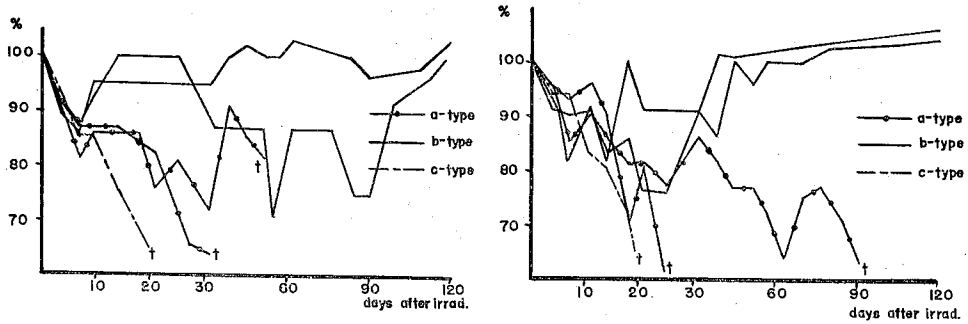


Fig. 2. Body weight changes in the mice irradiated lethally and treated with FL.

The cells were suspended in Tyrode's solution. The left figure shows the changes in HFL treatment in which the number of nucleated cells injected was 5 million. The right figure shows the changes in HFL treatment in which the number of nucleated cells injected was 10 million.

SFL cells protected the lethally irradiated mice from the early death better than the homologous bone marrow did.

**Body weight changes.** The changes of body weight in IFL and SFL transplantations are shown in Fig. 1. The changes in HFL transplantation are shown

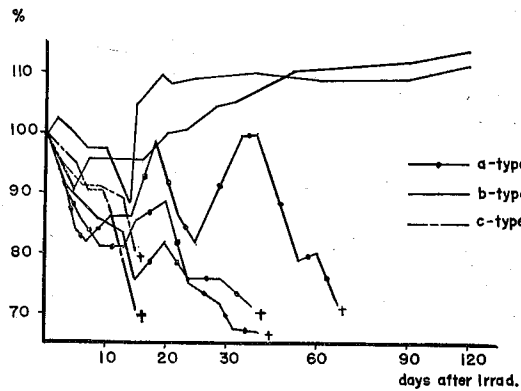


Fig. 3. Body weight changes in the mice irradiated lethally and treated with HFL.

The number of nucleated cells injected was 20 million. They were suspended in Tyrode's solution.

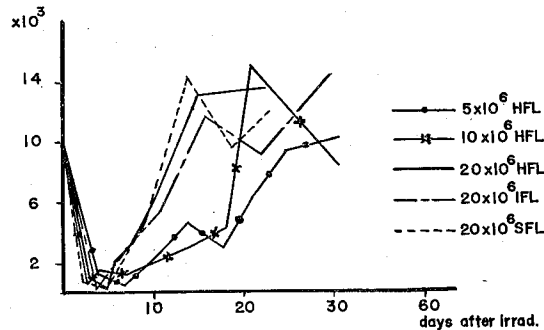


Fig. 4. Leucocyte count in gamma-irradiated mice treated with fetal liver cells.

in Fig. 2. The changes of body weight in  $20 \times 10^6$  HFL cells transplantation are shown in Fig. 3. The changes in IFL transplantation were similar to those in the isologous bone marrow transplantation. The lowest level of body weight after irradiation occurred at 7 days and then the body weight quickly increased to the preirradiation level at 18 days after irradiation. In other kinds of transplantation three types in the changes of body weight which were described in the homologous bone marrow transplantation were seen, namely, a-, b- and c-type<sup>32,33</sup>.

**Hematological findings. Leucocyte count.** The changes of leucocyte count in the experiments are shown in Fig. 4. The leucocyte count in the treated mice showed a rapid decrease to 500 to 1000 at 4 to 7 days. And then the count began to increase quickly or gradually and recovered to the normal level at 12 to 28 days. The HFL treatment in which the number of nucleated cells injected was  $5 \times 10^6$  or  $10 \times 10^6$  showed a retarded recovery.

**Erythrocyte count.** The changes of erythrocyte count in the treated ones are shown in Fig. 5. The lowest of the erythrocyte count occurred at 4 to 14 days, then the count increased gradually or quickly and recovered to the level of preirradiation at 10 to 30 days. However, the HFL treatments, in which the number of nucleated cells injected was  $5 \times 10^6$  or  $10 \times 10^6$ , showed a retarded recovery.

**Reticulocyte count.** The changes of reticulocyte count in the treated ones

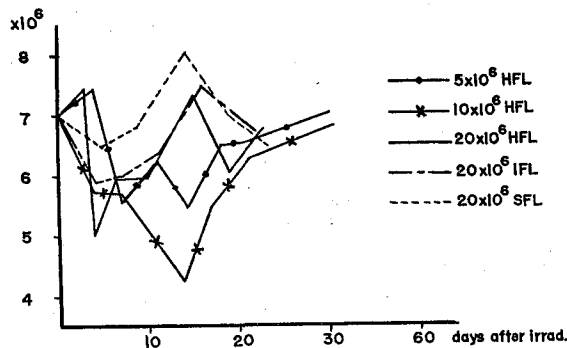


Fig. 5. Erythrocyte count in the gamma-irradiated mice treated with FL.

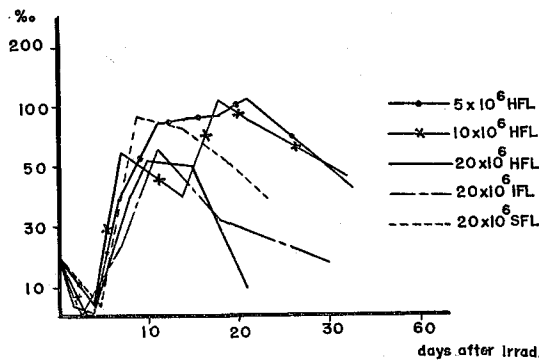


Fig. 6. Reticulocyte count in the gamma-irradiated mice treated with FL.

are shown in Fig. 6. The reticulocyte count in the treated mice decreased to zero by 2 to 6 days. It increased quickly or gradually starting at 4 days and showed one crisis of reticulocyte count. The peaks of the crisis appeared at 7 to 21 days. The count recovered to the preirradiation level at 18 to 45 days. Generally speaking, the times of appearance of the peaks and the recovery was retarded in the HFL treatments in which the number of nucleated cells injected was  $5 \times 10^6$  or  $10 \times 10^6$ .

**Platelet count.** The changes of platelet count are shown in Fig. 7. The count decreased to the level of  $250$  to  $200 \times 10^3$  at about 3 to 7 days and then increased rapidly or gradually to the level of preirradiation at 13 to 30 days. However, the HFL transplantation in which the number of nucleated cells injected was 5 million showed a slower recovery.

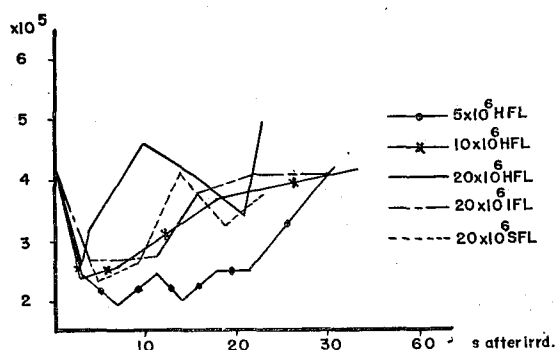


Fig. 7. Platelet count in the gamma-irradiated mice treated with FL.

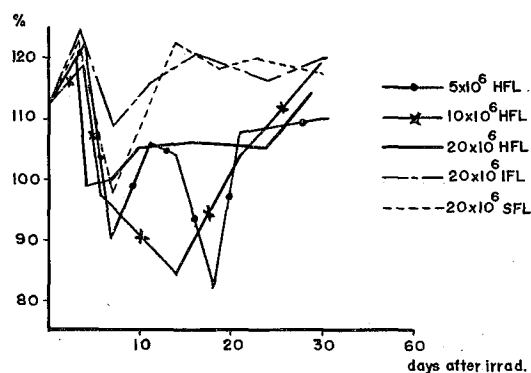


Fig. 8. Hemoglobin content in the mice irradiated lethally and treated with FL.

**Hemoglobin content.** Hemoglobin content was examined by the Sahli method. The changes of hemoglobin content are shown in Fig. 8. The hemoglobin content in the treated mice decreased to the level of 83–110% at 4 to 17 days after irradiation and then increased to the level of preirradiation at 7 to 35 days. The HFL transplantations in which the number of nucleated cells injected was 5 million and 10 million showed a retarded recovery.

**Histological findings.** The times of appearance of early regeneration and

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Table 2. The times of appearance of early regeneration and complete recovery in the hematopoietic tissues in gamma-irradiated mice treated with fetal liver.

Cell number	Bone marrow		Spleen				Thymus		Lymphnode	
	eg.	cp.	red eg.	pulp cp.	white eg.	pulp cp.	eg.	cp.	eg.	cp.
IFL	4	14	6	21	14	21	8	14	20	30
SFL	5	14	6	21	21	30	9	28	21	45
5×10 <sup>6</sup> HFL	4	14	6	25	—	50	—	50	—	50
10×10 <sup>6</sup> HFL	4	17	7	28	—	50	—	50	—	50
20×10 <sup>6</sup> HFL	4	14	7	21	—	70-90	10	70-90	—	70-90

eg. means early regeneration in the tissues in the mice which were lethally irradiated and treated with fetal liver.

cp. means complete recovery in the tissues in the mice which were lethally irradiated and treated with fetal liver.

complete recovery are shown in Table 2.

**Bone marrow.** The sternal and femoral bone marrow were examined. Early regeneration was initiated on the 4th to 6th day. Complete recovery was discernible on the 14th to 18th day. However, the complete recovery in the HFL transplantations in which the number of nucleated cells injected was 5 million and 10 million was slightly retarded by 3 to 7 days.

**Spleen.** The red pulp. Early regeneration in the red pulp was discernible on the 6th to 8th day and complete recovery was seen on the 21st to 25th day. The recovery to a normal cellularity was a little slower in the HFL transplantation in which the number of nucleated cells injected was 5 million or 10 million.

The white pulp. The early regeneration in the white pulp in IFL transplantation was discernible on the 14th day and the complete recovery was seen on the 28th day. In the transplantation other than IFL, complete recovery of the white pulp was retarded.

**Thymus.** Early regeneration in the thymus in IFL treatment was initiated on the 8th day and complete recovery was discernible on the 14th day. The early regeneration in the thymus after SFL treatment was initiated on the 28th day and a normal cellularity was discernible on the 35th day. Complete recovery in the thymus after HFL treatment was very much retarded.

The lymphatic tissues. Early regeneration in the lymphatic tissues was seen on the 14th day and complete recovery was seen on the 28th day IFL treatment. In other treatments, the times of appearance of early regeneration and complete recovery were very much retarded.

## 2. Some Observations on Long Term Preservation of Mouse Fetal Liver

This experiment was made to protect the lethally irradiated mice with the homologous fetal liver cells which were suspended in 15% glycerol-Tyrodé's solution or 10% DMSO-TC 199 solution and were stored at -80°C by slow freezing and used after fast thawing.

### I. Fetal liver suspended in 15% glycerol-Tyrodé's solution.

**Survival rates.** The survival rates in the experiment are shown in Fig. 9



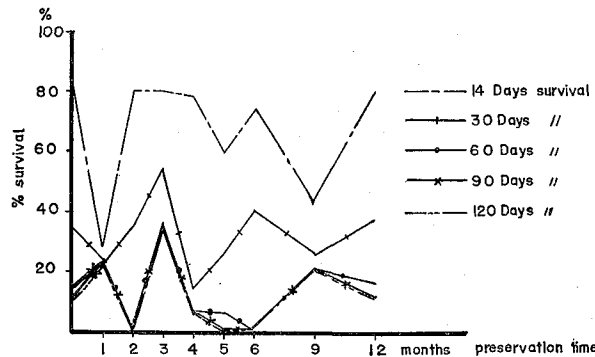


Fig. 9. Changes of percent survival in the mice irradiated lethally and treated with preserved HFL. The number of nucleated cells injected was 20 million. They were suspended in 15% glycerol-Tyrode's solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$ .

Table 3. Survival rates of gamma-irradiated mice treated with preserved HFL.

Preservation period	No. of experiment	Percent staining with eosin (%)	Number of survival (at days)						% Survival (at days)			
			Number of irradiated						21	30	60	90 120
Control	44	—	2/162	0/162	—	—	—	—	0	—	—	—
Fresh	5	23	27/33	18/33	11/32	5/32	4/32	4/32	54	34	15	12 12
30 days	2	28	4/14	3/13	3/13	3/13	3/13	3/13	23	23	23	23 23
60 days	2	26	9/11	5/11	4/11	0/11	0/11	0/11	45	35	0	0 0
90 days	2	28	9/11	7/11	6/11	4/11	4/11	4/11	63	54	36	36 36
120 days	2	26	11/14	4/14	2/14	1/14	1/14	2/14	28	14	7	7 7
150 days	2	35	9/15	7/15	4/15	1/15	0/15	0/15	46	26	6	0 0
180 days	2	45	11/15	10/15	6/15	0/15	0/15	0/15	66	40	0	0 0
270 days	2	53	7/16	4/15	4/15	3/14	3/14	3/14	26	26	21	21 21
360 days	3	91	16/20	9/20	7/18	3/17	2/16	2/16	45	38	17	12 12

The number of nucleated cells injected was 20 million. They were suspended in 15% glycerol-Tyrode's solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$ .

and Table 3. The number of nucleated cells injected was 20 million. The survival rates in the 30-day preserved HFL treatment were as follows: 28% at 14 days, 23% at 21 days, 23% at 120 days. The survival rates in a 360-day preserved HFL transplantation were as follows: 80% at 14 days, 45% at 21 days, 38% at 30 days, 17% at 60 days, 12% at 90 days, 12% at 120 days. It was found that the 360-day preserved HFL could protect the lethally irradiated mice as well as the fresh HFL did.

**Body weight changes.** The changes of body weight in the treatment are shown in Fig. 10. The three types described in the homologous bone marrow transplantation were observed. B-type was not seen in 60, 150 and 180 days preserved HFL transplantations.

**Hematological findings.** Leucocyte count. The changes of leucocyte count in the experiment are shown in Fig. 11. The count in the treated ones showed a

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rapid decrease at 4 to 9 days and then the count began to increase quickly or gradually and the time of recovery to the normal level was at 12 to 28 days. However, the time in the 360-day preserved HFL transplantation was later than those in the transplantation with HFL preserved for periods shorter than 360 days.

**Erythrocyte count.** The changes of erythrocyte count in the treated ones are shown in Fig. 12. The lowest level of the count occurred at 4 to 14 days and the count increased gradually or quickly and recovered to the level of preirradiation at 9 to 21 days.

**Reticulocyte count.** The changes of reticulocyte count in the treated ones are shown in Fig. 13. The count in the treated ones decreased to zero by 2 to 5 days.

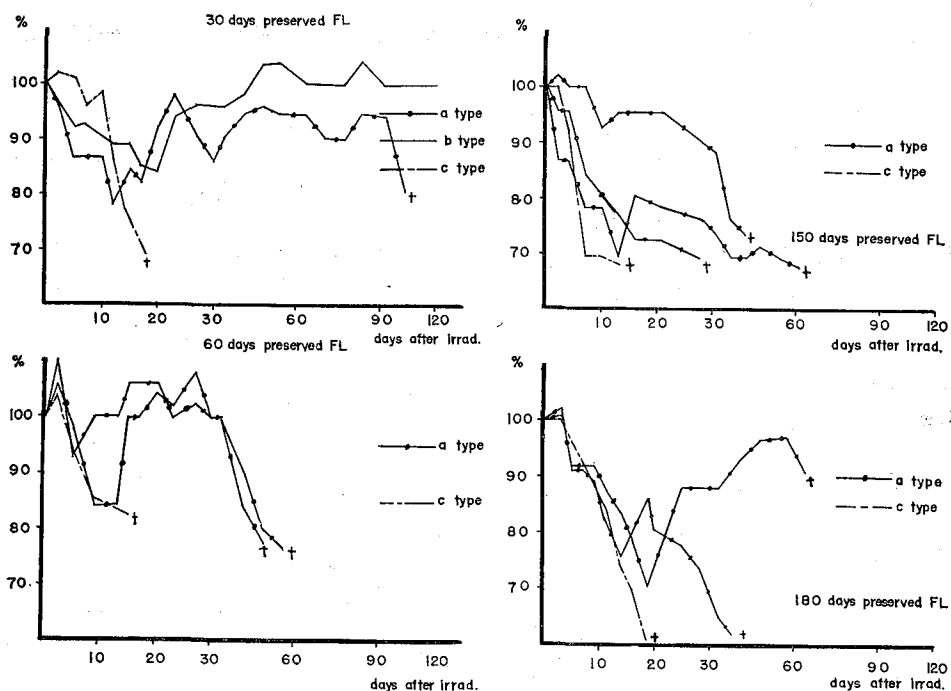


Fig. 10. Body weight changes in the mice irradiated lethally and treated with preserved HFL.

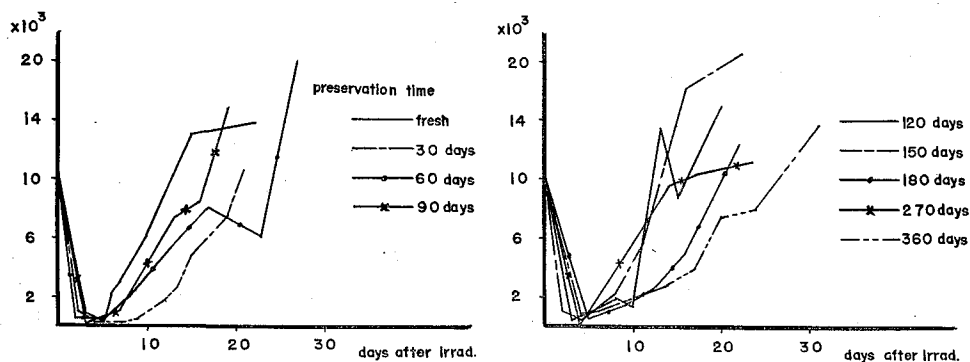


Fig. 11. Leucocyte count in the gamma-irradiated mice treated with preserved HFL.

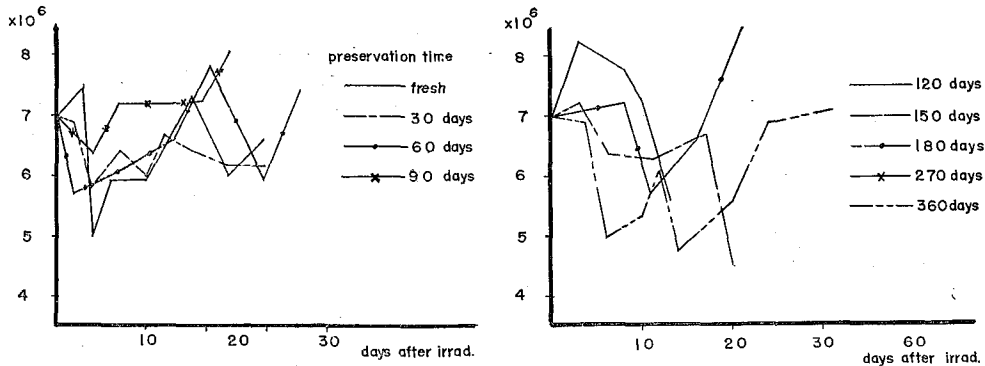


Fig. 12. Erythrocyte count in the gamma-irradiated mice treated with preserved HFL.

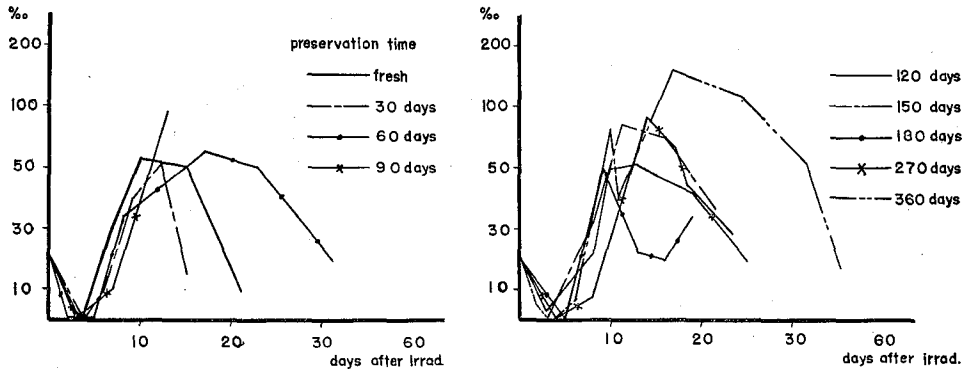


Fig. 13. Reticulocyte count in gamma-irradiated mice treated with preserved HFL.

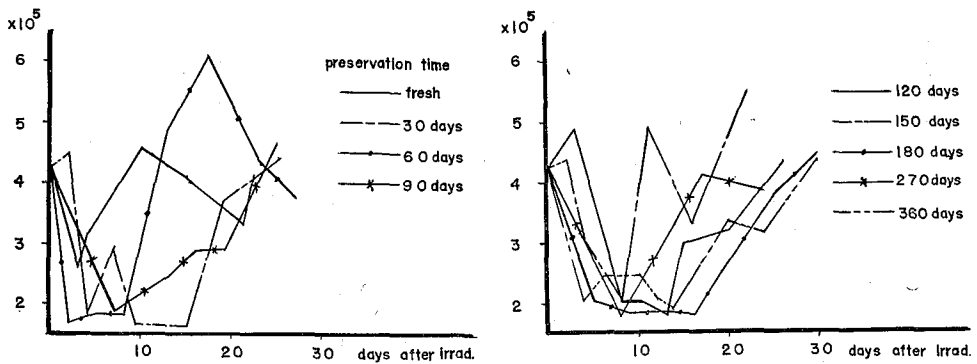


Fig. 14. Platelet count in the gamma-irradiated mice treated with preserved HFL.

It increased quickly or gradually at 6 days and showed one crisis. The peaks of the crisis were at 10 to 18 days.

**Platelet count.** The changes of platelet count are shown in Fig. 14. The count decreased to the level of 270 to  $100 \times 10^3$  at about 2 to 9 days. They increased rapidly or gradually and reached the level of preirradiation at 11 to 28 days.

**Hemoglobin content.** The changes of hemoglobin content are shown in Fig. 15.

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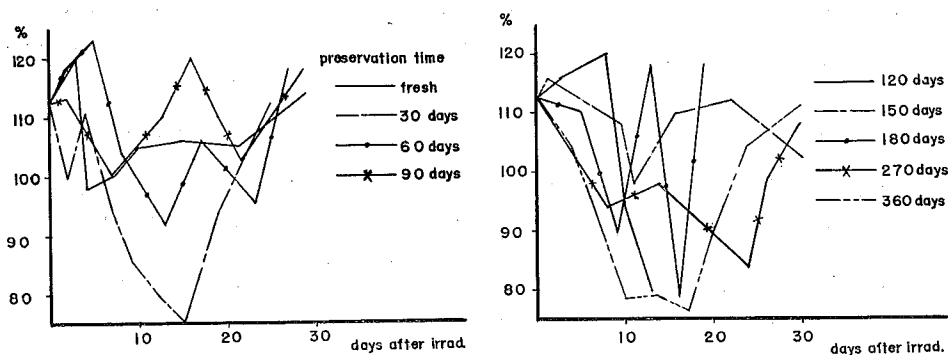


Fig. 15. Hemoglobin content in the gamma-irradiated mice treated with preserved HFL.

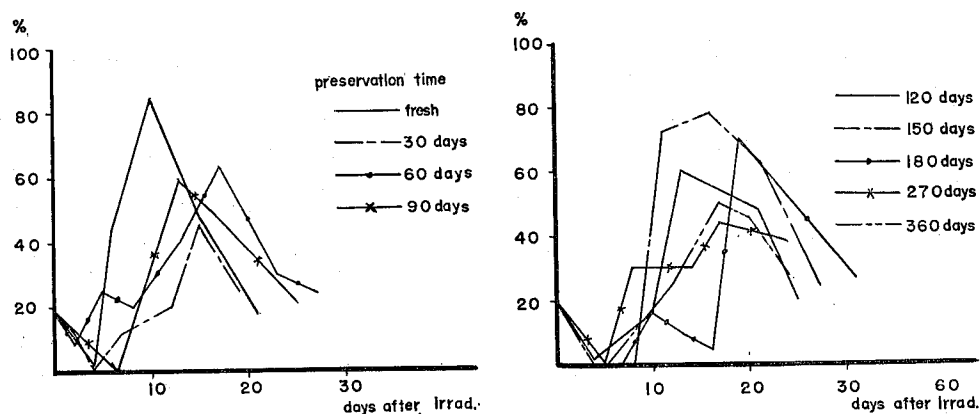


Fig. 16. The percentage of neutrophils in the gamma-irradiated mice treated with preserved HFL.

Table 4. The time of appearance of early regeneration and complete recovery in the hematopoietic tissues in gamma-irradiated mice treated with HFL.

Preservation period	Bone marrow		Spleen				Thymus		Lymphnode	
	eg.	cp.	red eg.	pulp cp.	white eg.	pulp cp.	eg.	cp.	eg.	cp.
Fresh	4	14	7	21	—	70-90	10	70-90	—	70-90
30 days	6	15	8	21	—	100	—	100	—	100
270 days	6	14	8	15	50	90	14	50	—	90
360 days	5	18	6	20	—	120	14	85	—	90

The number of nucleated cells injected was 20 million. They were suspended in 15% glycerol-Tyrode's solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$ .

eg. means early regeneration in the tissues in the mice which were lethally irradiated and treated with HFL.

cp. means complete recovery in the tissues in the mice which were lethally irradiated and treated with HBM.

The numbers in the Table show the days after irradiation.

The hemoglobin content in the treated ones decreased to the level of 70 to 99% Sahli at 4 to 15 days and then increased to the level of preirradiation at 14 to 28 days.

**Percentage of neutrophils.** The changes of percentage of neutrophils in the peripheral blood are shown in Fig. 16. The percentage of neutrophils is neutrophilic leucocytes/neutrophilic leucocytes + mononuclear cells. Peaks of the percentage appeared between 11 and 21 days and they recovered to the level of preirradiation between 21 and 35 days after irradiation.

**Histological findings.** The times of appearance of early regeneration and complete recovery are shown in Table 4. The times of appearance of early regeneration and complete recovery of the bone marrow and the red pulp of the spleen were almost the same as those in the fresh IFL transplantation. However, complete recovery in the lymphatic tissues came later than that in the IFL transplantation.

II. This experiment was made to protect lethally irradiated mice with HFL which was suspended in 10% DMSO-TC199 solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$ .

**Survival rates.** The survival rates in the experiment are shown in Fig. 17 and Table 5. The number of nucleated cells injected was 20 million. The survival rates in the 30-day preserved HFL transplantation were as follows: 68% at 14 days, 42% at 21 days, 40% at 30 days, 15% at 60 days, 15% at 120 days. The survival rates in the 270 days preserved HFL transplantation were as follow: 87% at 14 days, 56% at 21 days, 50% at 30 days, 43% at 60 days, 37% at 90 days, 37% at 120 days. The survival rates in the 360-day preserved HFL transplantation were as follows: 80% at 14 days, 20% at 21 days, 12% at 30 days, 8% at 60 days, 8% at 120 days.

**Body weight changes.** The body weight changes in the treatments are shown

Table 5. Survival rates of gamma-irradiated mice treated with preserved HFL.

Preservation period	No. of experiment	Percent staining with eosin (%)	Number of survival (at days)							% Survival (at days)				
			Number of irradiated											
			14	21	30	60	90	120	21	30	60	90	120	
Control	44	—	2/162	0/162	—	—	—	—	0	—	—	—	—	—
Fresh	5	23	27/33	18/33	11/32	5/32	4/32	4/32	54	34	15	12	12	
30 days	3	30	15/22	9/21	8/20	3/20	3/20	3/20	42	40	15	15	15	
60 days	2	55	7/15	4/15	1/15	1/15	1/15	1/15	26	6	6	6	6	
90 days	2	29	9/11	4/11	1/11	1/11	1/11	1/11	36	9	9	9	9	
120 days	2	47	4/16	2/16	0/16	0/16	0/16	0/16	12	0	0	0	0	
150 days	2	22	6/18	1/18	1/18	0/18	0/18	0/18	15	15	0	0	0	
180 days	2	43	7/17	2/17	2/17	2/17	1/17	1/17	11	11	11	5	5	
270 days	2	58	14/16	9/16	8/16	7/16	6/16	5/16	56	50	43	37	27	
360 days	3	74	12/25	5/24	3/24	2/24	2/24	2/24	20	12	8	8	8	

The number of nucleated cells injected was 20 million. They were suspended in 10% DMSO-TC199 solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$ .

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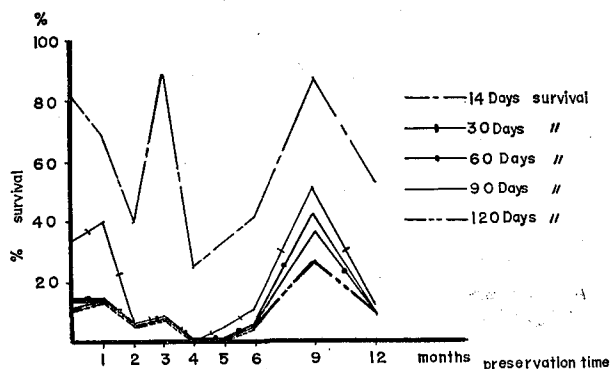


Fig. 17. Changes of percent survival in the mice irradiated lethally and treated with preserved HFL. The number of nucleated cells injected was 20 million. They were suspended in 10% DMSO-TC199 solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$ .

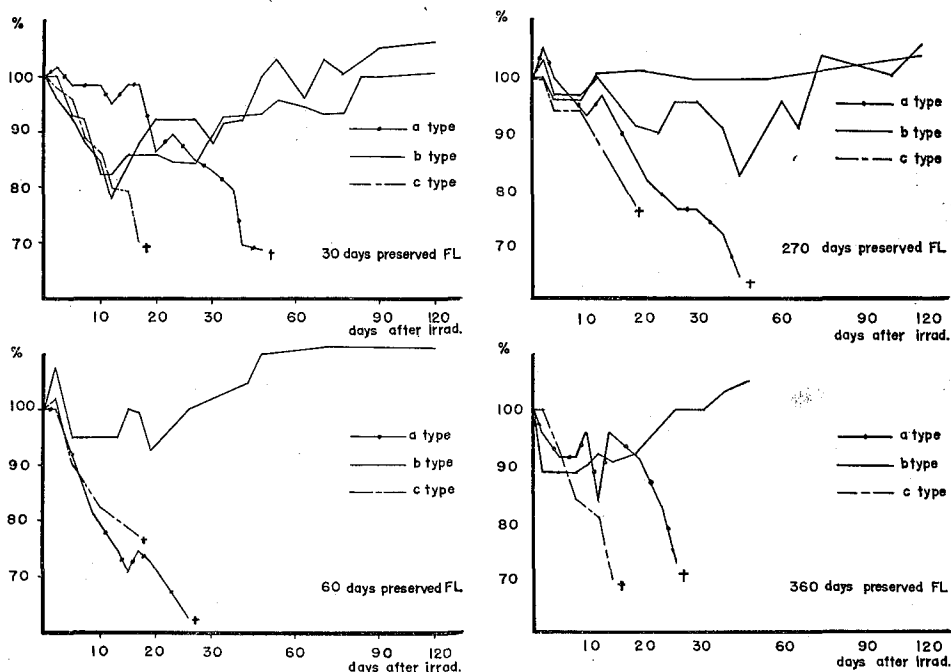


Fig. 18. Body weight changes in the mice irradiated lethally and treated with preserved HFL.

in Fig. 18. The three types described as previously were seen. B-type was not seen in the 120 and 150 days preserved HFL transplantation.

**Hematological findings.** Leucocyte count. The changes of leucocyte count in the experiment are shown in Fig. 19. The count in the treated ones showed a rapid decrease at 2 to 8 days and then the count began to increase quickly or gradually and the time of recovery to normal level was at 12 to 21 days.

Percentage of neutrophils. The changes of percentage of neutrophils in the peripheral blood are shown in Fig. 20. Peaks of the neutrophils count appeared

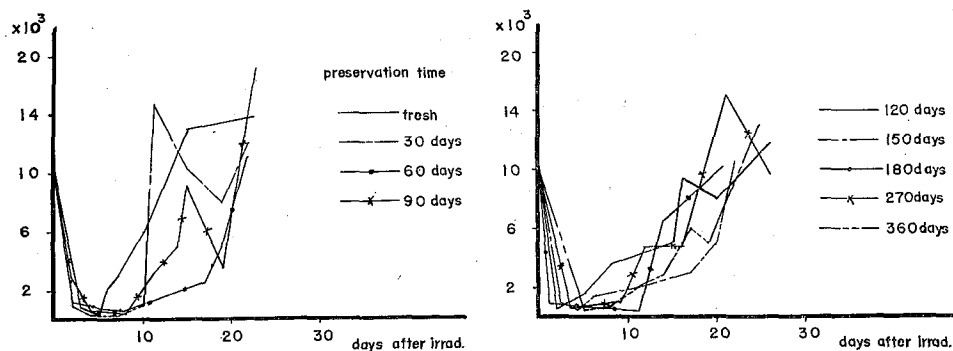


Fig. 19. Leucocyte count in the gamma-irradiated mice treated with preserved HFL.

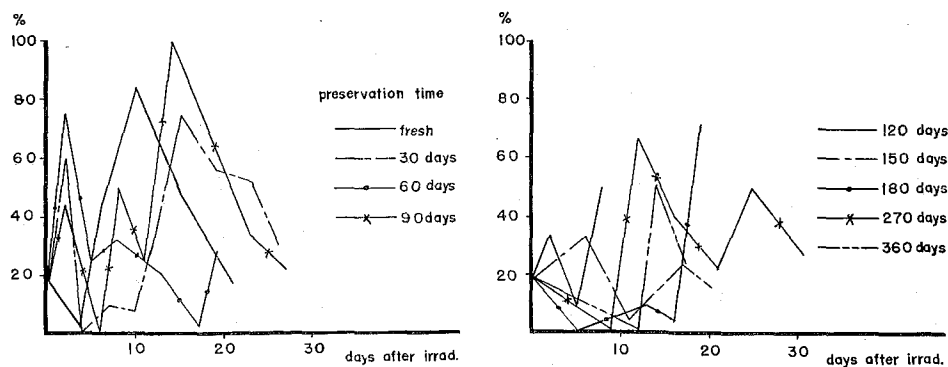


Fig. 20. The percentage of neutrophils in the gamma-irradiated mice treated with preserved HFL.

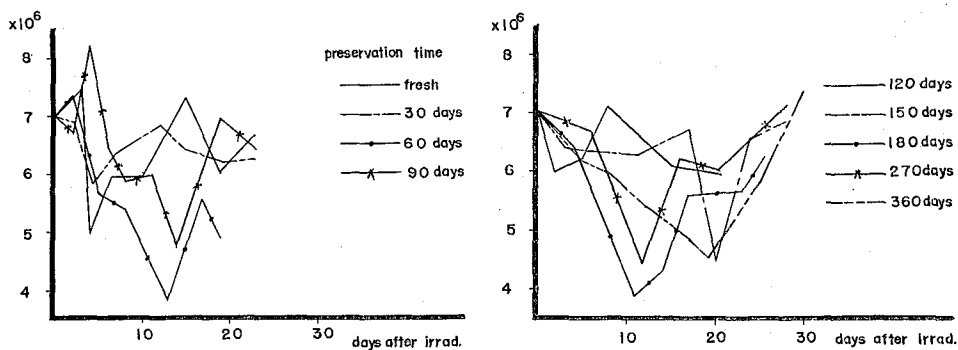


Fig. 21. Erythrocyte count in the gamma-irradiated mice treated with preserved HFL.

between 4 to 20 days. They returned to the preirradiation level between 12 and 30 days.

**Erythrocyte count.** The changes of erythrocyte count in the treated ones are shown in Fig. 21. The lowest level of the erythrocyte count occurred at 4 to 12 days and then the count increased quickly or gradually and recovered to the level of preirradiation at 14 to 28 days.

**Reticulocyte count.** The changes of reticulocyte count in the treated ones

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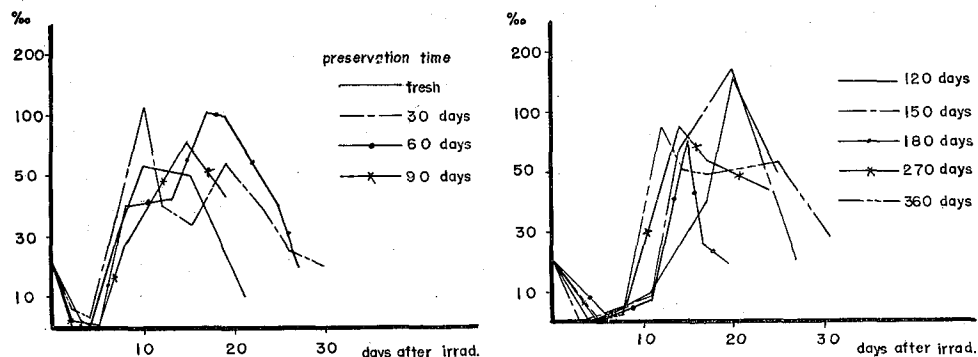


Fig. 22. Reticulocyte count in the gamma-irradiated mice treated with preserved HFL.

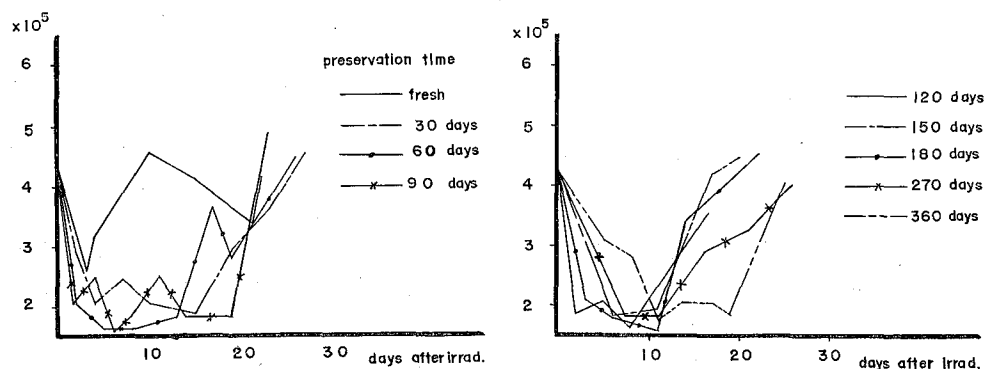


Fig. 23. Platelet count in the gamma-irradiated mice treated with preserved HFL.

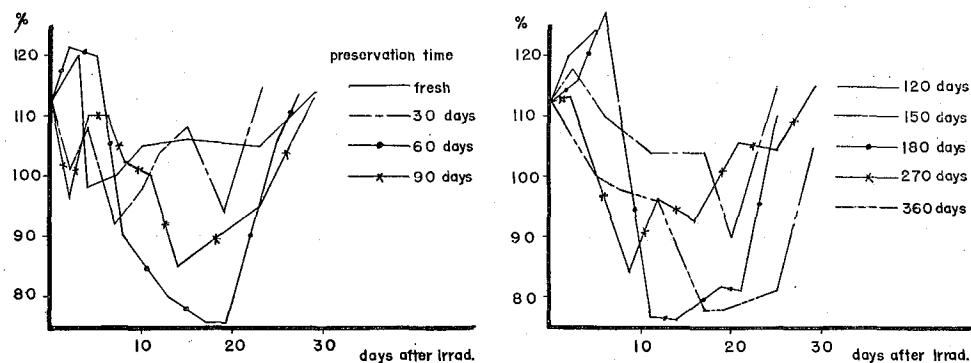


Fig. 24. Hemoglobin content in the gamma-irradiated mice treated with preserved HFL.

are shown in Fig. 22. The reticulocyte count in the treated ones decreased to nil by 2 to 5 days and then the count increased quickly or gradually to the level of preirradiation at 18 to 30 days.

**Platelet count.** The changes of platelet count are shown in Fig. 23. The count decreased to the level of 50 to 220 $\times 10^3$  at 5 to 12 days and then the count increased to the level of preirradiation at 15 to 25 days.

**Hemoglobin content.** The hemoglobin content are shown in Fig. 24. The



Table 6. The times of appearance of early regeneration and complete recovery in the hematopoietic tissues in gamma-irradiated mice treated with preserved HFL.

Preservation period	Bone marrow		red	Spleen		pulp	Thymus		Lymphnode	
	eg.	cp.		cp.	eg.		eg.	cp.	eg.	cp.
Fresh	4	14	7	21	—	70-90	10	70-90	—	70-90
30 days	4	14	4	21	—	75	14	75	—	75
150 days	6	15	9	21	—	115	9	115	—	115
270 days	4	14	7	20	—	70	20	70	—	70
360 days	5	20	8	21	—	—	14	—	—	—

The number of nucleated cells injected was 20 million. They were suspended in 10% DMSO-TC199 solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$ .

eg. means early regeneration in the tissues in the treated mice.

cp. means complete recovery in the tissues in the treated mice.

The numbers in the Table show the days after irradiation.

hemoglobin content decreased to the level of 70 to 95% Sahli at 9 to 18 days and then increased to the level of preirradiation at 21 to 28 days.

**Histological findings.** The times of appearance of early regeneration and complete recovery are shown in Table 6. The times of appearance of early regeneration and complete recovery of the bone marrow and the red pulp of the spleen were almost the same as those in the fresh HFL transplantation. However, complete recovery of the lymphatic tissues later than that in IFL transplantation.

## DISCUSSION

Mice exposed to a lethal dose of X radiation will survive the acute hematopoietic death when they are given a postirradiation inoculation of bone marrow or spleen. Treatment with isologous bone marrow produces excellent survivals, while homologous marrow affords only a temporary recovery; the mice succumb during a secondary phase of the irradiation syndrome. As for the late death, there have been two theories. Barnes and Loutit<sup>2)</sup> postulated an immunological response of marrow graft against the irradiated host. Makinodan and his coworkers<sup>3,4,5)</sup>, however, postulated a delayed immunological response of the host to the tissues of the foreign strain. There is an increasing body of evidences in support of this former theory as the explanation of the secondary phase. If there exists a graft versus host reaction when adult tissues is used, fetal hematopoietic tissue might not initiate such a reaction owing to its immunological immaturity (immunological nonreactivity).

The survival rates in the fresh homologous bone marrow transplantation when the number of nucleated cells injected was 5 million were as follows: 41% at 14 days, 18% at 21 days, 17% at 30 days, 10% at 60 days, 5% at 90 days after irradiation. The survival rates in the fresh homologous bone marrow transplantation when the number of nucleated cells injected was 10 million were as follows: 77% at 14 days, 58% at 21 days, 56% at 30 days, 30% at 60 days, 20% at 90 days and 16% at 120 days. As compared with the homologous bone marrow

### Preserved Hematopoietic Tissues for Treatment of Mice. (III)

transplantation, better survivals from acute radiation injury by the transplantation of HFL were obtained. The delayed death, however, occurred also in HFL treatments, as characterized by body weight loss, emaciation, and skin lesion. Uphoff<sup>9,10)</sup>, Lengerová<sup>34)</sup>, and Barnes et al<sup>12)</sup>, indicated that fetal liver in different genetic combinations was capable of altering delayed death in foreign bone marrow transplantation. But Urso et al<sup>14)</sup>, did not find a good long term survival in the mice transplanted with homologous fetal liver. Trentin<sup>35)</sup> reported that fetal blood-forming tissues were not superior to adult bone marrow in promoting long term survival after irradiation. In the author's experiments, the best survival was obtained in the transplantation with 5 million HFL cells. In the experiments, third trimester donors (15 to 19 days gestation) were used for the treatments. According to Tschetter et al.<sup>36,37)</sup>, the gestational age of donor fetal liver appeared to have no influence on the early death and fetal liver in the second trimester (12 to 14 days gestation) could protect the lethally irradiated mice from the late death better than that in the third trimester.

To preserve HFL cells for a long term, 15% glycerol-Tyrode's solution or 10% DMSO-TC 199 solution was used. The fetal liver cell suspensions were slowly frozen and used after fast thawing. Generally speaking, the preserved HFL could protect well the lethally irradiated mice from acute radiation death. However, delayed death occurred between the 21st to 120th day after irradiation. Hematologically, the speed of recovery to the preirradiation level was dependent on the number of nucleated cells injected and in some case, preservation period. The bone marrow and the red pulp of spleen did recover to preirradiation state as quickly as in the isologous fetal liver or bone marrow transplantation. Slow rate of recovery of the thymus, the white pulp of the spleen and the lymphatic tissue was recognized<sup>38)</sup>. They were filled with plasma cells and reticular cells on the 30th day.

### SUMMARY

1. The fresh homologous fetal liver cells supposed to be immunologically immature protected the lethally irradiated mice from acute radiation illness, but late death occurred. Treatment with 5 million HFL cells produced an excellent 120 day-survival.

2. The HFL suspended in 15% glycerol-Tyrode's solution or 10% DMSO-TC 199 solution and preserved for 30 to 360 days at  $-80^{\circ}\text{C}$  protected the lethally irradiated mice from acute radiation illness, but delayed death occurred. The 360-day preserved HFL could still protect the lethally irradiated mice.

### ACKNOWLEDGEMENTS

It is a great pleasure to thank Prof. G. Wakisaka, M. D. and Dr. H. Uchino, M. D. Yamagishi, and Dr. Adachi for their suggestions and advices in carrying out the study, and thanks are also extended to Mr. R. Katano Institute for Chemical Research of Kyōto University, for his kindness in frequent operating the Co<sup>60</sup> irradiation facility.

This work was supported by a grant-in-aid from the International Atomic Energy Agency 138/RB (1962-1964), to which thanks are also due.

### Explantation of Plates

Plate 1. Fetal liver of Na<sub>2</sub> strain mice of 19 days gestation. Immature hematopoietic cells are seen. H-E stain×400

Plate 2. Bone marrow ; 4 days after 900r gamma-irradiation and treatment with 360-day preserved HFL suspended in 15% glycerol-Tyrode's solution. This is early regeneration. The number of nucleated cells injected was 20 million. H-E stain×400

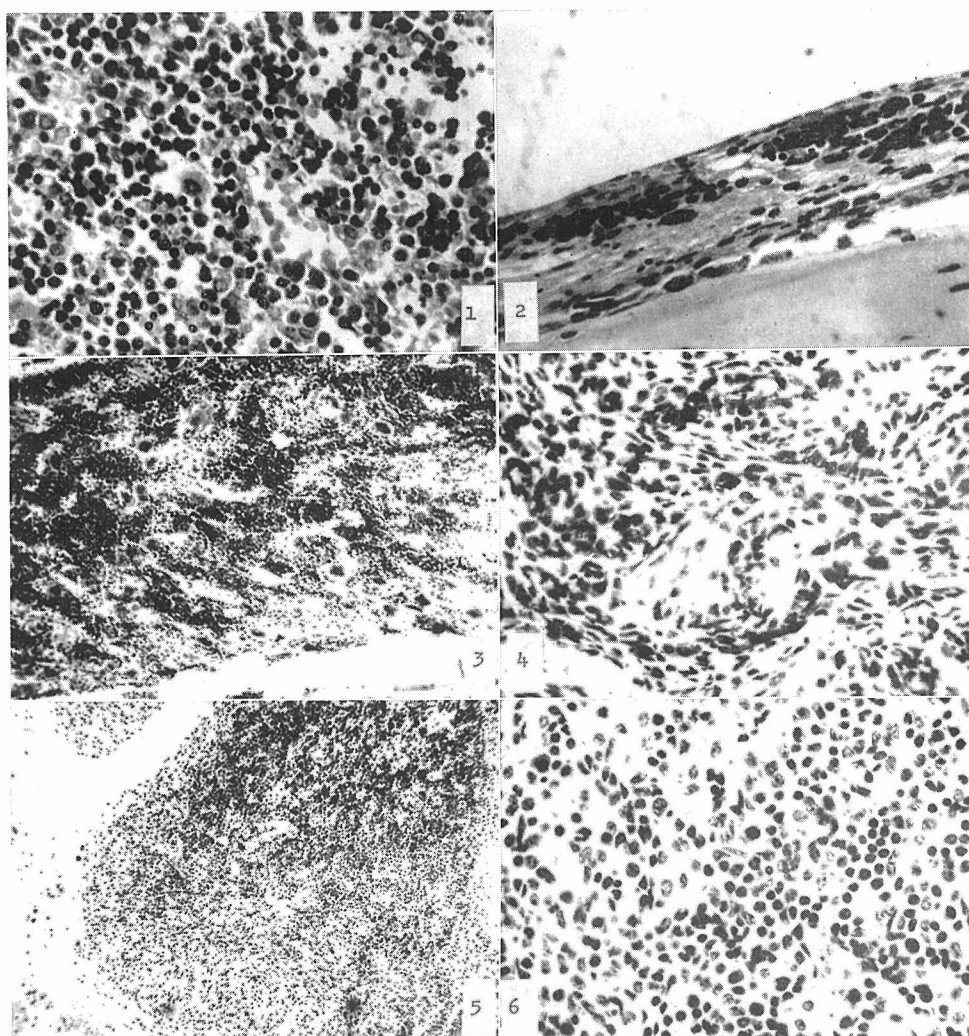
Plate 3. Bone marrow ; 14 days after 900r gamma-irradiation and treatment with fresh HFL. The number of nucleated cells injected was 5 million. The cellularity is normal. H-E stain×100

Plate 4. Spleen ; 60 days after 900r gamma-irradiation and treatment with 270-day preserved HFL suspended in 10% DMSO-TC199 solution. The white pulp is completely wasted. H-E stain×400

Plate 5. Thymus ; 75 days after 900r gamma-irradiation and treatment with 360-day preserved HFL suspended in 15% glycerol-Tyrode's solution. The number of nucleated cells injected was 20 million. A marked increase in number of thymocytes in the thymus. H-E stain×100

Plate 6. Brachial lymphnode ; 60 days after 900r gamma-irradiation and treatment with 270-day preserved HFL suspended in 10% DMSO-TC199 solution and the number of nucleated cells injected was 20 million. The lymphnode is wasted. H-E stain×400

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